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| (21) International Application Number: PCT/GB92/02256 (22) International Filing Date: 4 December 1992 (04.12.92) (30) Priority data: 9125891.3 5 December 1991 (05.12.91) GB (71) Applicant (for all designated States except US): THE INSTITUTE OF CANCER RESEARCH [GB/GB]; 17A Onslow Gardens, London SW7 3AL (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): HICKISH, Tamas [GB/GB]; CUNNINGHAM, David [GB/GB]; Department of Medicine, The Royal Marsden Hospital, Downs Road, Sutton, Surrey SM2 5PT (GB). (74) Agents: GOLDIN, Douglas, Michael et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5EU (GB). | | (81) Designated States: AU, CA, GB, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> |

(54) Title: ANTIVIRAL AGENT COMPRISING ANTISENSE OLIGONUCLEOTIDES COMPLEMENTARY TO THE BHRF1 GENE OF EPSTEIN-BARR VIRUS

(57) Abstract

The invention provides antisense oligonucleotides to the BHRF1 gene of Epstein-Barr virus (EBV), and the use of such oligonucleotides in methods of treatment of EBV infections.

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ANTIVIRAL AGENT COMPRISING ANTI-SENSE OLIGONUCLEOTIDES COMPLEMENTARY TO THE BHFR1 GENE OF EPSTEIN-BARR VIRUS.

The present invention relates to the use of antisense oligonucleotides in the treatment of Epstein-Barr Virus (EBV) infections, eg. EBV related tumors such as lymphomas.

- 5 The Epstein-Barr Virus is a human herpes virus which co-evolved with our species and frequently establishes a persistent asymptomatic infection of the circulating B-lymphocyte pool. The mechanism of virus persistence is not understood but given the limited life span of most B cells in vivo it seems most likely that EBV infected cells must gain
10 access to the long-lived memory B cell pool. (Klein 1989; Gregory C et al 1991). EBV is the causative agent of infectious mononucleosis and is associated with endemic African Burkitt's lymphoma, undifferentiated nasopharyngeal carcinoma, AIDS associated lymphoma and recently has been
15 found in association with Hodgkin's disease. (Zur Hausen et al 1970; Cleary M et al 1984; Herbst H et al 1991).

- EBV exists in two stages, the latent and lytic cycles. During the latent cycle the EBV genome is incorporated with
20 the host DNA and is non replicative whilst in the lytic cycle viral progeny are produced and this may result in lysis of the host cell.

- Recently the EBV genome has been identified in Hodgkin's disease cells. This has been made possible by developments
25 in the techniques of molecular biology enabling detection of rare copies of mRNA and DNA. This has been necessary as the malignant cells in Hodgkin's disease generally represent a small proportion of a given tumour biopsy with the remainder being an infiltrate of reactive cells. The EBV genome (and
30 its gene products) are most consistently associated with the nodular sclerosing and mixed cellularity subtypes of Hodgkin's disease.

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Hodgkin's disease can be sub-divided into four groups on the basis of morphological criteria. These are, lymphocyte predominant, nodular sclerosing, mixed cellularity and lymphocyte depleted. This sub-division translates into a different prognosis for each group. Overall the survival of patients with Hodgkin's disease is 80%. There remains a group of patients who are incurable either due to advanced or resistant disease. The curative treatment options range from radiotherapy (for localised disease) to cytotoxic chemotherapy with or without radiotherapy. A group of patients will demonstrate a pattern of persistent relapse and currently their best chance of cure lies with the high dose chemotherapy plus autologous bone marrow transplant. All therapies to date are nonspecific and are associated with toxicity. There is therefore a requirement for novel therapeutic options.

The EBV-induced early antigen complex (Henle et al 1971) is composed of restricted and diffuse components. BHRFI is an early antigen (restricted) protein and is encoded by the EBV DNA BamHI restriction fragment 1 (BHRFI). (Pearson et al 1987) BHRFI is a 17-KD protein, expressed at the interface between the latent and lytic cycles. In vitro studies indicate it is synthesised during the G1 phase of the cell cycle before the initiation of DNA synthesis. (Kocache et al 1990).

It has been found that BHRFI has 40% homology with the bcl2 gene which is deregulated in at least 90% of cases of follicular non-Hodgkin's lymphoma. Bcl2 is expressed at the inner mitochondrial membrane and deregulation appears to extend cell survival by interrupting programmed cell death (apoptosis) (Hockenbery et al 1990) BHRFI and bcl2 therefore may have functional equivalence.

We have now surprisingly found that BHRFI is localised to the mitochondria and that expression is found only in cells in

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the lytic cycle (i.e. producing intact virus) and that most of these cells are undergoing apoptosis.

While we do not wish to be bound by any one particular theory, we believe that EBV expresses BHRFI to extend survival of the host cell so as to maximise the yield of virus.

The sequence listing which follows the examples shows the sequence of the EBV genome in the region of the BHRFI gene together with a translation of the BHRFI gene. The sequence shown corresponds to nucleotides 54307 to 55020 of the EBV genome in Genbank release 26.0.

The present invention thus relates to a method of reducing the expression of BHRFI in cells infected with EBV. In particular, the present invention provides an antisense oligonucleotide which is selectively hybridizable to the BHRFI gene, compositions comprising such oligonucleotides including compositions of the oligonucleotides in admixture with a pharmaceutically acceptable carrier.

The invention further provides such an antisense oligonucleotide for use in a method of treatment or therapy of EBV infection of the human or animal body.

The invention also relates to a method of treatment of a human patient infected with EBV and in need of therapy which comprises administering to the patient an effective amount of an antisense oligonucleotide to the BHRFI gene.

Tumours containing the EBV genome are found in patients suffering from, Hodgkin's disease Burkitt's lymphoma, undifferentiated nasopharyngeal carcinoma, AIDS related lymphoma and angioimmunoblastic lymphadenopathy and thus the above method of treatment may be used to treat patients suffering from such conditions. Infectious mononucleosis is

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also associated with EBV.

5 The term "BHRFI gene" includes the entire transcribed region of this gene and also those 3' and 5' regions involved in the regulation of gene expression. The wild-type EBV produces a mRNA encoding the BHRFI gene product of about 1.9 kb in size. This RNA is encoded by nucleotides 53804 to about 55700 of the EBV geome in Genbank release 26.0. Methods for determining regulatory regions, eg. promoter regions are well known in the art.

10 Although oligonucleotides according to the invention will generally comprise a sequence which is exactly complementary to a part of the BHRFI gene, for example the sequence of Figure 1 (or the corresponding sequence of any other wild-type strain of EBV), some variation of the sequence may be
15 desirable or possible, provided that such variation allows the oligonucleotides to remain selectively hybridizable to their target region of the BHRFI gene. By "selectively hybridizable" it is meant that the sequence of the oligonucleotide will be more similar to the complement of its
20 intended target in the EBV genome than it is to any other nucleic acid sequence in the environment in which it is used. Usually, such an environment will contain the entire EBV genome, human genomic DNA, and the RNA expressed from both genomes. Occasionally, the environment may include other
25 human viruses, eg HIV-1 or HIV-2.

It will thus be understood that reference herein to oligonucleotides according to the invention encompasses such selectively hybridizable variants. Generally, the
30 oligonucleotide will not contain more than 1 to 5, eg 2, 3 or 4 differences between its sequence and that of the complement of the corresponding portion of the BHRFI gene against which it is directed.

Antisense oligonucleotides are short fragments of DNA or RNA

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(oligonucleotides) which by virtue of their nucleotide sequence are designed to hybridise to DNA or RNA targets and thereby block transcription or translation of a specific gene. If expression of the target gene expression is essential for survival of the malignant cell population then gene blockade would result in cell death (reviewed in Helene 1990).

From the foregoing paragraph, it is to be understood that an oligonucleotide designed to target the RNA transcribed from a gene will be antisense to the plus strand of the EBV genome (ie. the plus strand as shown in SEQ ID. No. 1). However, an oligonucleotide designed to be antisense to the DNA of EBV may be antisense to either strand of the double stranded EBV genome. For example, translation or transcription factors which recognise specific sequences of either the plus or minus strands of EBV could be targeted by supplying oligonucleotide comprising such sequences which will then compete for such factors with the native EBV.

It is however generally preferred that an oligonucleotide of the invention will be complementary to at least a portion of the mRNA transcript of the EBV BHRFI gene.

For in vivo activity antisense oligonucleotides will require modifications in the molecules backbone and possibly additions at either the 5' or 3', or both, ends of the molecule. This is to (1) achieve resistance to degradation by nucleases such as DNAases, (2) enhance the potency of the molecule and (3) to enhance uptake of the oligonucleotide by cells. This is an area of intense research and development. Currently the technology exists to produce sufficient quantities of modified oligonucleotides for therapeutic use (Biosystems Reporter 1991). A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5'

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ends of the molecule. Further modified oligonucleotides within the scope of the invention include polyamide nucleic acids. For the purposes of the present invention, it is to be understood that references to oligonucleotides herein
5 include oligonucleotides modified by any method available in the art in order to enhance their in vivo activity or lifespan.

Additions to the 5' end include mono-, di- or tri-phosphate moieties.

10 Further, it will be clear to those of skill in the art that although specific oligonucleotides described herein are represented in standard notation, ie., by the letters a, c, g, t (or u) to indicate the base of the nucleotide, written in a 5' to 3' direction (unless specified to the contrary),
15 these sequences may be modified as described above. In addition, other types of oligonucleotides which contain nucleotide residues which are N-glycosides or C-glycosides of purine or pyrimidine bases, or modified purine or pyrimidine bases, are included within the scope of the invention and are
20 encompassed by the term oligonucleotide. Examples of modified nucleotides include 2'-deoxy-2-aminoadenosine, 2'-deoxy-inosine and 2'-deoxy-xanthosine. Further Examples of modified oligonucleotides which may be used in the present invention are disclosed in PCT/US91/03680.

25 Thus, oligonucleotides according to the present invention will typically be from 6 to 30 bases in length, eg. from 8 to 25 or 15 to 25, preferably from 10 to 20 or 21, eg. 14, 15, 16, 17, 18 or 19 bases.

The oligonucleotides may be directed against any part of the
30 BHRFI gene. One region of the gene of interest is the 5' translated region, starting at or within a few nucleotides (eg. 3 to 12) of the initiation "atg" codon. However, antisense oligonucleotides to other regions of the gene may

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be used, eg. from the 5' untranslated region, anywhere in the translated region including the 3' translated region or the 3' untranslated region.

We have found that in vitro that oligonucleotides may be used effectively at a concentration of about 5 μ M, eg from 500nM to 500 μ M, eg. from 1 μ M to 50 μ M. For in vivo use the amount of oligonucleotide which may be used will ultimately be determined by a physician, taking into account the requirements of a patient, although it is expected that it will be necessary and desirable to deliver concentrations of oligonucleotides in the order of those mentioned above to the environment of infected cells.

Cells of the human body infected with EBV may be treated in vitro and reintroduced into the body. This is a technique which finds application in, for example, autologous bone marrow transplants. Accordingly, the invention provides a method for the treatment of EBV-induced proliferative diseases of white blood cells such as Hodgkin's disease which comprises removing the bone marrow of a patient in need of such treatment, treating the marrow with an oligonucleotide according to the invention and reimplanting the marrow into the patient following treatment of the patient to destroy proliferating tumour cells which remain in the patient.

Oligonucleotides according to the invention may be a single specific sequence or a mixture of different sequences.

Oligonucleotides according to the invention may be mixed with carriers or diluents suitable for delivery to a patient. Oligonucleotides may be formulated as injectable compositions, or in solid tablet form for oral administration. Other formulations, eg, for nasal, topical, rectal or vaginal administration may be used. The oligonucleotides may be formulated in unit dosage forms for single or multiple daily administration. Injectable

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compositions may be formulated to be delivered as a continuous infusion.

Further, oligonucleotides according to the invention may be formulated to enhance or target their delivery. They may be encapsulated in liposomes or attached to antibodies (or fragments thereof) suitable to target cells with EBV infection. BHRFI is potentially an ideal target for the antisense approach since this gene would be unique to EBV infected cells and furthermore these cells often display epitomes which may enable antibody directed targeting of the oligonucleotides to the malignant cell population. The oligonucleotides may also be linked to other carriers, eg proteins or other nucleic acids.

One antisense oligonucleotide according to the invention is a 17mer directed against the 5' translated region of BHRFI. It has the following sequence: 5' ct tgt tga ata ggc cat 3'. Other oligonucleotides according to the invention include oligonucleotides encompassed by this sequence, eg:

5' t tgt tga ata ggc cat 3';
5' tgt tga ata ggc cat 3'; and
5' gt gta ata ggc cat 3'.

The following examples illustrate the invention.

Example 1.

Cell Line and Culture Conditions

To explore the function BHRFI we have examined an EBV-genome positive cell line, B95.8, using low temperature embedding immunoelectron microscopy.

B95.8 was cultured in serum free medium (RPMI 1641). Under these conditions expression of BHRFI is enhanced. On day 0 cell in log phase growth were seeded at 2×10^5 /ml in total volume of 1ml in 24 well microtitre plates. Oligonucleotides

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(sense and antisense) were added at a concentration of 5 μ M.

Oligonucleotides

Phosphodiester oligonucleotide (17 mer) sequence antisense to the start codon and the 14 bases 3' was assayed with the sense sequence serving as the control. These oligonucleotides were also assayed on an EBV negative cell line.

sense 5' atg gcc tat tca aca ag 3'

(start codon underlined)

antisense 5' ct tgt tga ata ggc cat 3'

Cells were counted on days 1, 2 and 3 using a haemocytometer.

Results

Three experiments were performed. On day 3 the surviving fraction of cells cultured with the antisense oligonucleotide was 50% of that of the controls (either no oligonucleotide or sense oligonucleotide). The antisense oligonucleotide was inactive in the EBV negative cell line.

Example 2.

Phosphorothioate oligonucleotides were made with the following sequences:

Antisense: 3' ctcgttctaccggataagtt 5'

Sense: 5' gagcaagatggcctattcaa 3'

16.5 μ m of the oligonucleotides were added to cells and the cells cultured as described in Example 1. Figure 1 shows the fraction of cells surviving over a four day period compared to untreated control cells (surviving fraction = 1).

References

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SEQUENCE LISTING

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 720 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Epstein-Barr virus

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 76..648
 (D) OTHER INFORMATION: /product= "BHRFI gene product"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
 (B) LOCATION: 1..720
 (D) OTHER INFORMATION: /note= "Corresponds to 54301..55020 of EBV sequence of GenBank release 26.0"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | |
|--|-----|
| ACGTGGTCT AACTTGAATT TTTTGGTTTT CTAGTCCCT CTTAATTACA TTTGTGCCAG | 60 |
| ATCTTGTAGA GCAAG ATG GCC TAT TCA ACA AGG GAG ATA CTG TTA GCC CTG | 111 |
| Met Ala Tyr Ser Thr Arg Glu Ile Leu Leu Ala Leu | |
| 1 5 10 | |
| TGT ATA CGG GAC AGT CGT GTG CAT GGA AAT GGT ACC CTG CAT CCT GTG | 159 |
| Cys Ile Arg Asp Ser Arg Val His Gly Asn Gly Thr His Pro Val | |
| 15 20 25 | |
| TTG GAG CTA GCA GCA AGA GAA ACA CCT CTC CGC CTT TCG CCA GAG GAC | 207 |
| Leu Glu Leu Ala Ala Arg Glu Thr Pro Leu Arg Leu Ser Pro Glu Asp | |
| 30 35 40 | |
| ACT GTA GTT CTG CGT TAT CAT GTG TTG CTT GAG GAG ATA ATT GAA CGA | 255 |
| Thr Val Val Leu Arg Tyr His Val Leu Leu Glu Glu Ile Ile Glu Arg | |
| 45 50 55 60 | |
| AAT TCA GAG ACA TTT ACA GAA ACT TGG AAC AGA TTT ATA ACA CAC ACC | 303 |
| Asn Ser Glu Thr Phe Thr Glu Thr Trp Asn Arg Phe Ile Thr His Thr | |
| 65 70 75 | |
| GAA CAT GTG GAT CTG GAT TTT AAC TCA GTA TTT TTA GAG ATA TTT CAC | 351 |
| Glu His Val Asp Leu Asp Phe Asn Ser Val Phe Leu Glu Ile Phe His | |
| 80 85 90 | |
| CGT GGA GAC CCA AGC CTT GGG CGC GCG TTG GCC TGG ATG GCC TGG TGC | 399 |
| Arg Gly Asp Pro Ser Leu Gly Arg Ala Leu Ala Trp Met Ala Trp Cys | |
| 95 100 105 | |
| ATG CAT GCC TGC AGG ACA TTG TGT TGT AAC CAG TCT ACT CCT TAC TAT | 447 |
| Met His Ala Cys Arg Thr Leu Cys Cys Asn Gln Ser Thr Pro Tyr Tyr | |
| 110 115 120 | |
| GTT GTG GAC CTG TCA GTT CGT GGG ATG TTA GAA GCC AGC GAA GGC CTG | 495 |
| Val Val Asp Leu Ser Val Arg Gly Met Leu Glu Ala Ser Glu Gly Leu | |
| 125 130 135 140 | |

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| | |
|---|-----|
| GAT GGT TGG ATT CAT CAA CAG GGC GGC TGG TCT ACA TTA ATT GAA GAC | 543 |
| Asp Gly Trp Ile His Gln Gln Gly Gly Trp Ser Thr Leu Ile Glu Asp | |
| 145 150 155 | |
| AAC ATT CCT GGA TCC AGA AGG TTT AGC TGG ACT TTG TTT CTT GCT GGA | 591 |
| Asn Ile Pro Gly Ser Arg Arg Phe Ser Trp Thr Leu Phe Leu Ala Gly | |
| 160 165 170 | |
| CTG ACT TTG AGT CTG TTA GTT ATA TGT AGT TAT TTA TTT ATC TCC AGA | 639 |
| Leu Thr Leu Ser Leu Leu Val Ile Cys Ser Tyr Leu Phe Ile Ser Arg | |
| 175 180 185 | |
| GGA AGA CAC TAATCTATAC ATTTTCTCAG CACTTTATAT GAATCAGGGT | 688 |
| Gly Arg His | |
| 190 | |
| CATTGGGCGCT GCGGGGAAC T GAGCCAGTAG GA | 720 |

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 191 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

| | |
|---|--|
| Met Ala Tyr Ser Thr Arg Glu Ile Leu Leu Ala Leu Cys Ile Arg Asp | |
| 1 5 10 15 | |
| Ser Arg Val His Gly Asn Gly Thr Leu His Pro Val Leu Glu Leu Ala | |
| 20 25 30 | |
| Ala Arg Glu Thr Pro Leu Arg Leu Ser Pro Glu Asp Thr Val Val Leu | |
| 35 40 45 | |
| Arg Tyr His Val Leu Leu Glu Glu Ile Ile Glu Arg Asn Ser Glu Thr | |
| 50 55 60 | |
| Phe Thr Glu Thr Trp Asn Arg Phe Ile Thr His Thr Glu His Val Asp | |
| 65 70 75 80 | |
| Leu Asp Phe Asn Ser Val Phe Leu Glu Ile Phe His Arg Gly Asp Pro | |
| 85 90 95 | |
| Ser Leu Gly Arg Ala Leu Ala Trp Met Ala Trp Cys Met His Ala Cys | |
| 100 105 110 | |
| Arg Thr Leu Cys Cys Asn Gln Ser Thr Pro Tyr Tyr Val Val Asp Leu | |
| 115 120 125 | |
| Ser Val Arg Gly Met Leu Glu Ala Ser Glu Gly Leu Asp Gly Trp Ile | |
| 130 135 140 | |
| His Gln Gln Gly Gly Trp Ser Thr Leu Ile Glu Asp Asn Ile Pro Gly | |
| 145 150 155 160 | |
| Ser Arg Arg Phe Ser Trp Thr Leu Phe Leu Ala Gly Leu Thr Leu Ser | |
| 165 170 175 | |
| Leu Leu Val Ile Cys Ser Tyr Leu Phe Ile Ser Arg Gly Arg His | |
| 180 185 190 | |

CLAIMS

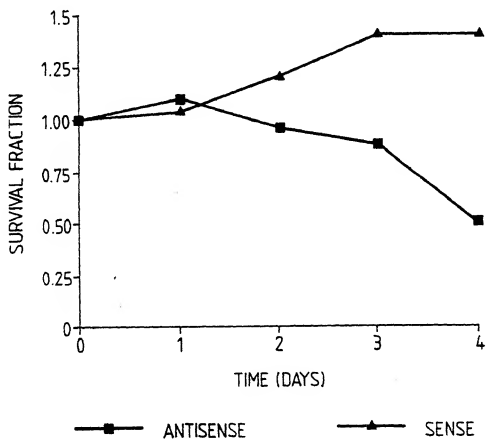
1. An antisense oligonucleotide which is selectively hybridizable to the BHRFI gene.
2. An oligonucleotide according to claim 1 which is to either
5 strand of the sequence of SEQ ID. No. 1.
3. An oligonucleotide according to any one of the preceding claims which is directed to the translated region of the BHRFI gene.
4. An oligonucleotide according to any one of the preceding
10 claims which is directed against the coding strand of the sequence of SEQ ID No.1.
5. An oligonucleotide according to any one of the preceding claims which is from 10 to 25 nucleotides in length.
6. An oligonucleotide according to claim 5 which is from 15
15 to 20 nucleotides in length.
7. An oligonucleotide according to any one of the preceding claims which is a DNA oligonucleotide.
8. An oligonucleotide according to any one of the preceding
20 claims which has a modified backbone and/or 5' end and/or 3' end.
9. An oligonucleotide according to claim 8 which has a triphosphate moiety at its 5' end.

10. An oligonucleotide according to any one of the preceding claims which has the following sequence:
5' cttgttgaataggccat 3'; or
5' aacttatccggtagaacgag 3'.
- 5 11. A composition comprising the oligonucleotide of any one of the preceding claims in admixture with a pharmaceutically acceptable carrier.
- 10 12. An antisense oligonucleotide as defined in any one of claims 1 to 11 for use in a method of treatment or therapy of EBV infection of the human or animal body.
13. An antisense oligonucleotide as defined in any one of claims 1 to 11 for use in a method of treatment of EBV infected cells in autologous bone marrow transplants of the human or animal body.
- 15 14. A method of treatment of an EBV infection of a patient which comprises administering to a patient in need of treatment an effective amount of an antisense oligonucleotide to the BHRFI gene.
- 20 15. A method according to claim 14 wherein the patient is suffering from one or more conditions from the group consisting of infectious mononucleosis, Hodgkin's disease Burkitt's lymphoma, undifferentiated nasopharyngeal carcinoma, AIDS related lymphoma and angioimmunoblastic lymphadenopathy.

16. A method for the treatment of EBV-induced proliferative diseases which comprises removing the bone marrow of a patient in need of such treatment, treating the marrow with an oligonucleotide according to the invention and reimplanting the marrow into the patient following treatment of the patient to destroy proliferating tumour cells.

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Fig.1.



SUBSTITUTE

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| I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶ | | |
| According to International Patent Classification (IPC) or to both National Classification and IPC | | |
| Int.Cl. 5 C12Q1/70; | C07H21/02; | C07H21/04; A61K31/70 |
| II. FIELDS SEARCHED | | |
| Minimum Documentation Searched ⁷ | | |
| Classification System | Classification Symbols | |
| Int.Cl. 5 | C12Q ; | C07H ; C12N ; A61K |
| Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸ | | |
| III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ | | |
| Category ¹⁰ | Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹² | Relevant to Claim No. ¹³ |
| X | PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 85, June 1988, WASHINGTON US pages 3678 - 3682 P.J. AUSTIN ET AL. see abstract see page 3680, right column, line 29 - page 3681, left column, line 8 --- | 1, 3-7, 12, 13 |
| A | VIROLOGY vol. 160, no. 1, September 1987, NEW YORK, USA pages 151 - 161 G.R. PEARSON ET AL. cited in the application see abstract see " introduction " and " discussion " --- | 1 |
| | | -/- |
| <p>¹⁰ Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "A" document member of the same patent family</p> | | |
| IV. CERTIFICATION | | |
| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report | |
| 11 MARCH 1993 | 24.03.93 | |
| International Searching Authority | Signature of Authorized Officer | |
| EUROPEAN PATENT OFFICE | LUZZATTO E.R. | |

III. DOCUMENTS CONSIDERED TO BE RELEVANT

(CONTINUED FROM THE SECOND SHEET)

| Category * | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No. |
|------------|--|-----------------------|
| A | WO,A,9 112 811 (ISIS PHARMACEUTICALS, INC.) 5 September 1991 see page 5, line 16 - page 6, line 18 see page 18, line 22 - page 20, line 14; claims --- | 1,2 |
| P,X | WO,A,9 204 903 (THE UNITED STATES OF AMERICA) 2 April 1992 see the whole document, especially fig. 2 and claims ----- | 1-3 |

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

GB 9202256
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This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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11/03/93

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